



Prospective Randomized Controlled Study to Compare the Outcome of Standard 4-Port Laparoscopic Cholecystectomy with Single-Incision Laparoscopic Cholecystectomy in Patients with Gallstone Disease

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Abstract

Four-port laparoscopic cholecystectomy (LC) is the gold standard treatment for symptomatic gallbladder disease. To reduce the invasiveness of standard four-port laparoscopic cholecystectomy, single-incision laparoscopic cholecystectomy (SILC) has come about an attractive option for the performance of laparoscopic cholecystectomy. There have been no studies on SILC from Indian subcontinent. The present study was designed to compare the outcomes of SILC with the standard four-port LC in a prospective randomized controlled trial. All patients with symptomatic gallstone disease were evaluated between May 2012 and April 2014. Patients were randomized to either standard four-port laparoscopic cholecystectomy (LC group) or single-incision laparoscopic cholecystectomy (SILC group). Demographic profile, preoperative and intraoperative variables, postoperative complications, hospital stay, and pain scores were recorded. WHO-QOL BREF was used for quality of life analysis. Patients were followed up at regular intervals, and satisfaction scores were recorded. Statistical analysis was done using STATA 12 and p value < 0.05 was considered significant. Out of 94 patients, 90 received the intended treatment, and four cases in SILC group were converted to standard four-port cholecystectomy. The demographic profile and preoperative WHO-QOL BREF scores were comparable between the two groups. Severity of adhesions, successful dissection of Calot's triangle, ergonomics, and overall level of difficulty were also comparable. Operation time was significantly higher in SILC group, but the learning curve was seen to be achieved after 30–35 cases. There was no significant difference in the incidence of immediate postoperative and chronic pain over a mean follow-up of 6 months except for pain score during normal activity in immediate postoperative period which was significantly higher in SILC group. Overall complication rate was significantly higher in SILC group, however the incidence of SSI was not found to be significant among the two groups. There was one case of transient bile leak and one case of intra-abdominal bleeding due to slippage of cystic artery clip. Postoperative quality of life outcomes were similar in the two groups. Although not significant, patients with SILC group had higher cosmetic score compared with the LC group. In conclusion, this study shows that SILC is a safe and feasible with a higher rate of complications but comparable cosmetic and QoL outcomes when compared with standard 4-port cholecystectomy.

Keywords Gallstone disease · Laparoscopic cholecystectomy · SILC · QoL

Introduction

Laparoscopic cholecystectomy (LC) is the gold standard treatment for benign and symptomatic gallbladder disease [1, 2].

The standard laparoscopic cholecystectomy has been associated with a conversion rate of 0.2% [3], biliary complication rate of 0.26 to 0.6% [4, 5], and bowel injury rate of 0.14 to 0.35% [4, 5]. There has been a continuous endeavor to reduce the invasiveness and thus wound-related complications of LC, and simultaneously improve the cosmetic outcomes of LC [6]. Various natural orifices including the transgastric, transrectal, and transvaginal route have been used as access without much success [7, 8].

To reduce the invasiveness of standard four-port cholecystectomy, single-incision laparoscopic cholecystectomy (SILC) has also become an attractive option for the

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performance of laparoscopic cholecystectomy [9, 10]. There have been numerous trials which include case studies and case series which have demonstrated the feasibility and efficacy of this single-incision technique, however risk/benefit ratio of SILC is not well established. There has been concern about the risk of increased complications in this procedure. In a study by Allemann et al. [11], the incidence of bile duct injury was 0.4%, and in a study by Jørgensen et al. [12], the incidence of incisional hernia was 2%. The current published literature is not yet conclusive on efficacy, safety, short- and long-term outcome. There are studies concluding lower pain and better cosmetic results in SILC [9], and others concluded higher pain and no significant difference in cosmetic results [10]. The present prospective randomized controlled study was designed to compare efficacy, safety, success, and cost of SILC related to surgical outcome, acute and chronic pain, cosmetic outcome, and quality of life with that from standard four-port standard LC.

Research question. Is SILC better than standard 4-port cholecystectomy in terms of safety, success, cost, cosmetic outcome, and quality of life outcomes?

Materials and Methods

This study was conducted as a superiority trial in a single surgical unit at a tertiary care hospital, after clearance from the Institute Ethics Committee from 3 November 2012 to 31 July 2014. The trial was registered in Clinical Trial Registry of India (CTRI/2012/10/004155). The study was a single blinded study.

All consecutive patients presenting with symptomatic gallstone disease undergoing surgery for gallstones were included in the study and prospectively randomized into two groups based—group 1, standard four-port LC; group 2, SILC.

Patients who do not give consent for participation in the study, age < 18 years, morbid obesity (BMI \geq 30 kg/m²), previous history of acute cholecystitis, pancreatitis or cholangitis, unfit for general anesthesia, uncorrectable coagulopathy, significant co-morbidities like coronary artery disease, asthma, COPD, uncontrolled diabetes mellitus (HbA1c > 8), hypertension and previous malignancy, and patients requiring other concomitant procedures were excluded from the study.

Work-up of Patient

Patients with gallstone disease underwent detailed clinical examination and laboratory investigations. The demographic profile like age, sex, and history of the illness; like duration of symptoms, history of jaundice, history of cholangitis, number of episodes of biliary colic, and any history of acute cholecystitis or pancreatitis were noted in a prestructured pro forma. Routine hemogram and biochemistry was done. A transabdominal ultrasound was done, and findings were noted

including number of stones, size of stone, gallbladder wall thickness, status of CBD, and other relevant findings.

Assuming that complications in the two modalities are the same (blood loss) with no difference in cosmetic outcome, the operative time with significant difference (41.3 ± 12 min in SILC and 35.6 ± 16 min in four port (p value = 0.01)) [13] was used with a α value of 5% and power 80%; a sample size of 86 was calculated. Assuming a dropout rate of 10%, 100 patients were enrolled with 50 patients in each group.

Only those patients who gave written consent for both the procedures were randomized. Randomization was done using computer-generated random numbers using block randomization technique and were divided into blocks of eight using sealed envelopes to ensure concealed allocation. The study was done according to CONSORT [14] guidelines for randomized trials and ICMR/GCP guidelines for study on human subjects.

Group 1 patients underwent a standard four-port cholecystectomy and group 2 underwent a SILC using SILS™ port (COVIDIEN™) placed at the umbilicus.

Primary Outcome Measure

The primary outcome measure was defined as the successful removal of gallbladder by intended modality, *i.e.*, by standard four-port laparoscopic cholecystectomy in group 1 and single-incision laparoscopic cholecystectomy in group 2.

Secondary Outcome Measures

1. Intraoperative parameters
2. Morbidity
3. Operative time
4. Level of difficulty. Level of difficulty was graded by the cumulative scores of the following parameters by the surgeon depending on his judgment at the end of the procedure
5. Hospital stay
6. Pain score. Pain was recorded on visual analog scale (VAS) at preoperative period, immediate postoperative period, 24 h after operation, or at the time of discharge whichever was early and during follow-up at 1 week, 6 weeks, 3 and 6 months, and further grouped into different level of activity, *i.e.*, at rest, normal activities, strenuous exercise, and in last 24 h.
7. Quality of life. All patients were given WHO quality of life assessment pro forma (WHOQOL-BREF) in English and Hindi after admission to hospital. The same pro forma was also given at 3 months follow-up and findings were compared.
8. Patient satisfaction score. Cosmesis was analyzed by patient's satisfaction score on surgery and on scar at 1 week which was labeled on Likert's verbal rating scale (VRS).

Follow-Up Assessment

Postoperatively, the patients were followed up at 1 week, 6 weeks, 3 months, and up to 6 months. The presence of pain and its severity was recorded on VAS, condition of the wound, and any other problem were noted on the prestructured pro forma.

Statistical Analysis

Data was collected and managed using Microsoft Excel (Microsoft Office Standard 2010 version 14.0, Seattle, WA, USA, Annexure VII) and analyzed by using STATA 12 (College Station, TX, USA). The unpaired Student’s *t* test was used to determine the significance of difference between two independent groups for continuous variables. For skewed

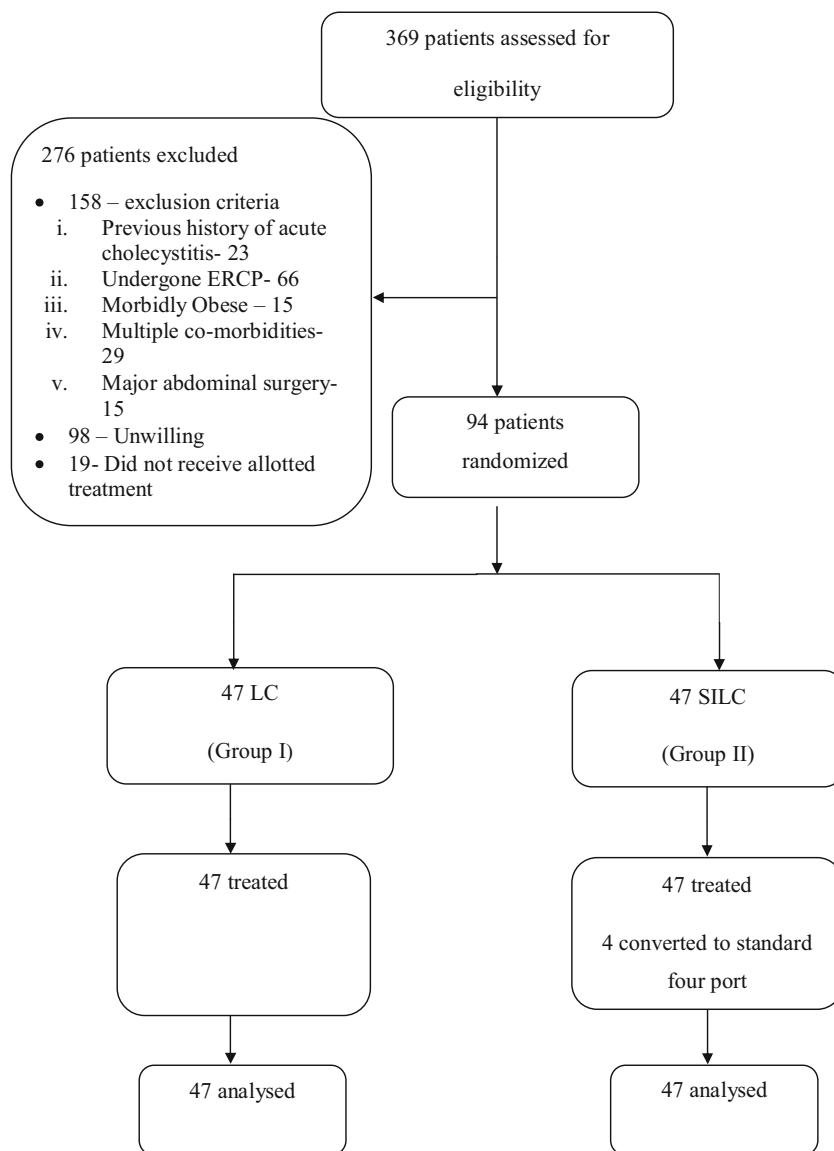
data, a corresponding nonparametric test, the Mann-Whitney *U* test was applied. For qualitative data, the chi-square test was used to compare the groups. Intent to treat analysis was performed to compare the outcomes. A *p* value < 0.05 was considered significant.

Results

Demography and Patient Parameters

A total of 369 patients with symptomatic gallstone disease were screened for inclusion in the study in a single surgical unit between November 2012 and July 2014. After exclusion, 94 (25.5%) patients with symptomatic gallstone disease were included in the study (CONSORT diagram, Fig. 1). They were divided into two groups.

Fig. 1 CONSORT diagram



Group 1 included 47 patients who underwent standard four-port LC, and group 2 included 47 patients who underwent SILC.

Both groups were comparable in terms of demographic, preoperative clinical, biochemical as well as radiological profile. (Table 1).

Intra-Operative Findings

Laparoscopic cholecystectomy could be completed successfully in 100% patients in LC group as compared with 91.5% in SILC group. The success rate in LC group was 8.5% higher than SILC group with a success ratio of 1.09 (Table 2). However, this clinically apparent difference did not translate into a statistically significant difference (p value = 0.12).

Table 1 Comparison of demographic, clinical, and radiological profile

Baseline characteristics	LC ($n = 47$)	SILC ($n = 47$)
Age (years)		
Mean \pm SD	34.15 \pm 8.3	32.5 \pm 9.14
Range	(18–52)	(18–51)
Sex		
Male	15 (31.9%)	12 (25.5%)
Female	32 (68.1%)	37 (78.7%)
BMI (kg/m^2)		
Mean \pm SD	22.10 \pm 2.14	22.36 \pm 2.38
Range	(19.65–27.5)	(18.04–28.25)
Previous abdominal surgery	14 (29.8%)	10 (21.3%)
Hemoglobin (gms/dl)		
Mean \pm SD	12.85 \pm 1.33	13.02 \pm 1.38
Bilirubin (mg/dl)		
Mean \pm SD	0.55 \pm 0.21	0.58 \pm 0.20
SGOT (IU)		
Mean \pm SD	20.46 \pm 5.8	20.63 \pm 5.62
SGPT (IU)		
Mean \pm SD	21.12 \pm 4.47	21.18 \pm 4.5
Alkaline phosphatase (IU)		
Mean \pm SD	180.2 \pm 32.47	179.29 \pm 32.75
TLC (mm^3)		
Mean \pm SD	6586.4 \pm 1079.62	6694.54 \pm 1161.98
USG findings		
CBD diameter (mm)		
Mean \pm SD	4.18 \pm 0.80	4.1 \pm 0.88
Stone size (mm)		
Mean \pm SD	5.34 \pm 2.54	5.36 \pm 2.48
GB		
Wall thickness (mm)		
Mean \pm SD	0.293 \pm 0.1	0.278 \pm 0.1
Solitary stone	12 (27.7%)	15 (30%)

Both the groups were comparable in terms of intraoperative parameters. Both groups were comparable in terms of intra-abdominal adhesions. Both the groups had comparable incidence of bile and stone spillage (8 (17.0%) vs 6 (12.7%), p value = 0.5 and 3 (6.4%) vs 5 (10.6%), p value = 0.4). Significantly larger number of patients in the LC group required either dilatation of epigastric port or extension of skin incision for delivery of gallbladder (15 vs 0, p value = 0.01).

There was also apparent difference between two groups in terms of level of difficulty with 8.5% (4 out of 47) procedures in SILC group graded as difficult by the operating surgeon as compared with none in LC group. However, this difference did not translate into a statistically significant difference (p value = 0.12) (Table 2).

The mean operative time was significantly higher in the SILC group when compared with LC group ((35.5 \pm 7.03 min vs 28.2 \pm 6.4 min (p value = 0.01)) (Table 2). On further analyzing the operative time by applying “moving averages,” we found that the operative time was longer in SILC group compared with LC group in the initial 30 cases only and there after become comparable (Fig. 2).

Postoperative Parameters

Hospital Stay

The average hospital stay for the study group was 26.04 h, and both groups were comparable in terms of hospital stay (25.21 \pm 5 h in LC group vs 27.06 \pm 7 h in SILC group (p value = 0.9)) (Table 2).

Complications

Patients undergoing SILC had significantly higher complication rate (3 (6.4%) in LC group vs 10 (21.3%) in SILC group (p value = 0.020)). Three patients in LC group had SSSI and were managed with local wound care and oral antibiotics. In SILC group, 6 (12.7%) patients had SSSI, 2 (4.2%) patients had seroma, 1 patient had transient bile leak, and 1 patient developed bleeding in the immediate postoperative period and had to be re-explored. On re-exploration, bleeding was found from slippage from cystic artery clip which was ligated, and the patient made an uneventful recovery (Table 2).

Pain Outcome (Table 3)

The pain score was analyzed on VAS. The preoperative pain score in SILC group was comparable with that in LC group (0.41 \pm 0.49 vs 0.40 \pm 0.49 (p value = 0.092)). Also, the pain scores in the postoperative period and at follow-up were comparable between the LC and SILC group with no patient in either group having pain at 6 weeks and 3 months. However, there was significantly lower pain in the LC group in the

Table 2 Comparison of Outcome

	LC (n = 47)	SILC (n = 47)	p value
Primary outcome			
Successful	47 (100%)	43 (91.5%)	0.12
Unsuccessful	0	4(8.5%)	
Secondary outcome			
Bile Spillage	6 (12.7%)	8 (17.0%)	0.77
Stone Spillage	5 (10.6%)	3 (6.4%)	0.72
Blood loss (ml)			0.8
Mean ± SD	18.7 ± 9.17	18.29 ± 8.55	
Extension of sheath incision	7 (14.9%)	0%	0.02
Adhesions			
Flimsy (score 1)	23(48.9%)	19(40.7%)	0.27
Moderate (score 2)	18(38.2%)	22(46.8%)	0.28
Dense (score 3)	0	1 (2.1%)	0.89
Calot's triangle			0.4
Not difficult (score1)	47 (100%)	44 (93.6%)	
Difficult (score 2)	-	1 (2.12%)	
Very difficult (score 3)	-	2 (4.2%)	
Ergonomics			1
Comfortable (score 1)	47 (100%)	47 (100%)	
Dissection of gallbladder			1
Not difficult	47 (100%)	47 (100%)	
Level of difficulty			0.12
Not difficult (score 0–4)	47 (100%)	43 (91.5%)	
Difficult (score 5–8)	0	4 (8.5%)	
Operative time (minutes)			0.01
Mean ± SD	28.2 ± 6.4	35.5 ± 7.03	
Postoperative parameters			
Hospital stay (hours)			0.9
Mean ± SD	27.06 ± 7	25.21 ± 5	
Complications		10 (21.3%)	
Total	3 (6.4%)	6 (12.8%)	0.020
SSI	3 (6.4%)	2 (4.2%)	0.21
Seroma	NA	1 (2.1%)	-
Bile leak	NA	1 (2.1 %)	-
Intra-abdominal bleeding	NA	10 (21.3 %)	-

immediate postoperative period as compared with SILC group at normal activity, but the difference was not seen at 24 h and at 1 week.

Quality of Life

The quality of life was assessed using the WHOQOL-BREF (field trial version) which are divided into four domain scores, i.e., physical health, psychological, social health, and environment, and these domains were further divided into individual facets. Both the groups were comparable in terms of quality of life (QoL) with higher mean scores in SILC group compared with LC group, which was however not statistically significant (Fig. 2).

Cosmetic Outcome

Overall patients in both groups were well satisfied by the cosmetic outcome of the procedure. In LC group, patient satisfaction score was 3.73 which was marginally lower than SILC group (3.58). But this did not translate into statistically significant difference (p value = 0.24) (Fig. 2).

Discussion

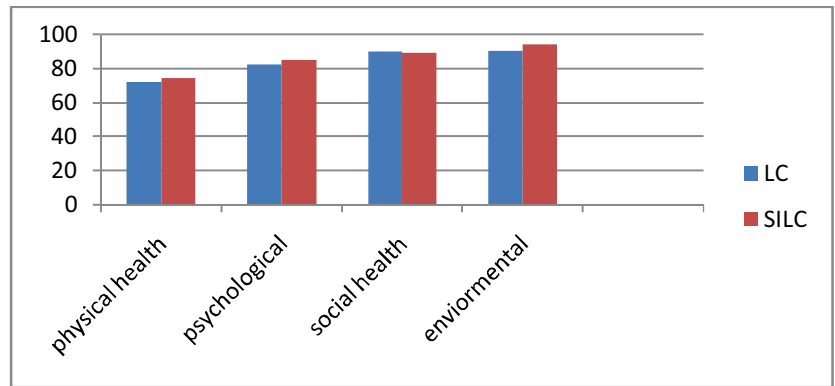
LC is the gold standard treatment for gallstone disease. The technique of laparoscopic cholecystectomy has been standardized, and the outcome of the patients following LC is almost stable with a conversion rate of 0.2% [3], biliary complication rate of 0.26 to 0.6% [4, 5], and bowel injury rate of 0.14 to 0.35% [4, 5]. Majority of the morbidity related to pain, wound complications, and cosmetic outcomes are related to the access sites for LC. The wound-related complications still account for almost 2.3% of the overall morbidity [6]. There has been a continuous endeavor to reduce the invasiveness and thus wound-related complications of LC, and also improve the cosmetic outcomes of LC. Various natural orifices including the transgastric, transrectal, and transvaginal route have been used, however their results have not been satisfactory with a question on their safety and reproducibility of the technique [7, 8].

SILC is associated with single-incision and is considered less invasive to standard four-port laparoscopic cholecystectomy. Thus, it has become an attractive option for performing cholecystectomy. There has been tremendous development in this field in the last decade and various access devices and instruments have been developed to carry out SILC. Though there are few studies which have demonstrated the efficacy of SILC, the pros and cons associated with SILS cholecystectomy are still not clear. It has been documented that there is risk of complications in this procedure. Allemann et al. [11] has documented 0.4% incidence of bile duct injury in their series, whereas Jørgensen et al. [12] has noted 2% incidence of incisional hernia development following SILC. The present literature is not conclusive on safety and long-term outcomes of this procedure. There have been no studies on SILC from Indian subcontinent.

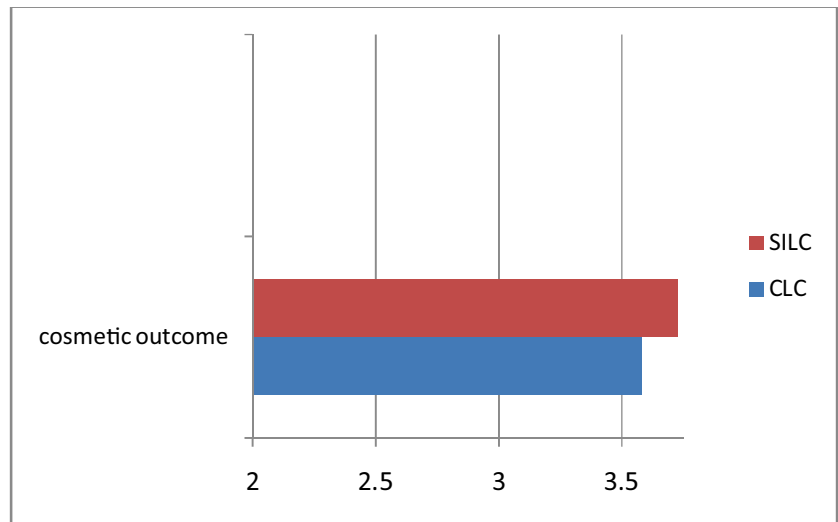
We have studied the level of difficulty experienced by the surgeon while performing the two procedures. Although the level of difficulty was not different between the two procedures, but the surgeons were not very comfortable in performing the SILC as compared with LC. The major difficulty was felt at the time of dissection around the Calot's triangle and during the application of the liga clips for ligation of cystic duct and cystic artery. The surgeons felt that difficulty was because of problem in visualization, and the instruments were in coaxial line with CBD which was a major

Fig. 2 QOL, cosmetic outcomes, and learning curve comparison

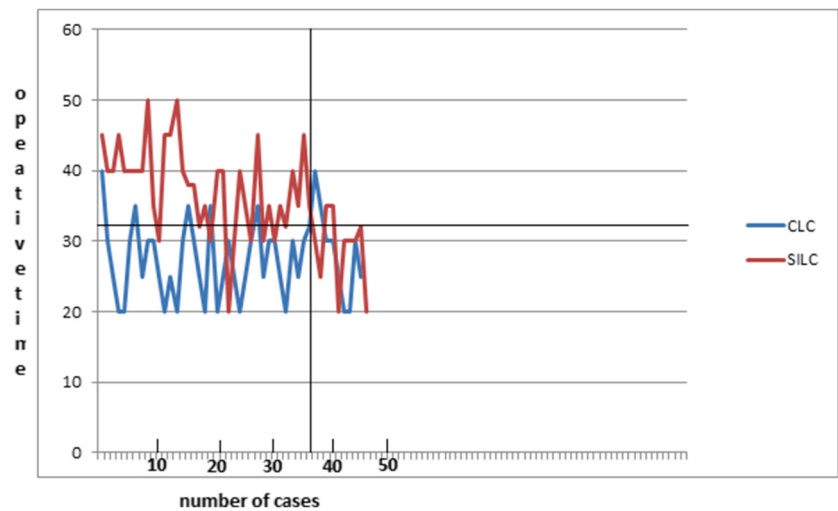
Comparison Quality of Life in LC and SILC group at 3 months



Comparison of Cosmetic Outcome



Operative time trend in LC and SILC group



compared with cases 26 to 50, the average length of time was 60 min ($p < 0.05$). In the present randomized study, also the mean operative time was significantly higher in the SILC group when compared with LC group (35.5 min vs 28.2 min (p value = 0.01).

Postoperative pain is a surrogate marker of procedure-related trauma. Outcome of SILC in terms of postoperative pain is variable in literature. Meta-analysis of various studies suggest no difference in postoperative pain in both the techniques [15, 18]. However, studies included in these meta-analysis were often heterogenous, and there was no uniformity in measure of pain as well as activity level at the time of pain measurement. Some studies have measured pain at rest while some during activity. Our study is unique in this regards as we have analyzed pain at various level of daily activity. In our study, there was significantly lower pain in the LC group at normal activity in the immediate postoperative period as compared with SILC group (4.92 vs 5.2 (p value = 0.0068)). As in the present trial, similar finding were also reported in two randomized trials. These randomized trials also reported significantly increased postoperative pain with no difference on follow-up in patients allocated to SILC [19, 20]. Increased pain may be a result of additional torque applied in SILC to allow for triangulation and exposure, or it may be a result in increased incision length at a single location.

QOL of patients has become a central evaluation parameter for chronic illness and morbidity. There is paucity of data in literature addressing the comparison of quality of life in standard four-port cholecystectomy and single-incision laparoscopic cholecystectomy. In a study by Hauters et al., QOL was assessed with the Gastrointestinal Quality of Life Index (GIQLI) questionnaire, and postoperative GIQLI scores were not significantly different between the two groups. In the current study, both groups were comparable in baseline quality of life, preoperatively, postoperatively at 3 months. Thus, SILC did not offer any advantage over standard four-port cholecystectomy in terms of cosmesis as well as QOL.

The main limitation of this study is lack of long-term follow-up; hence, the incidence of port site hernia, a major complication after SILC, could not be commented upon.

In conclusion, this study shows that SILC is a safe and feasible with a higher rate of complications, but comparable cosmetic and QOL outcomes when compared with standard 4-port cholecystectomy.

Author Contribution VKB and MCM were the operating surgeons and made substantive intellectual contributions in this study, including acquisition of data, interpretation and drafting/editing of the manuscript, and have given final approval of the version to be published. AK has given substantive intellectual contributions in this study and helped in the interpretation and drafting/editing of the manuscript, and has given final approval of the version to be published. SG made substantive contributions to this study, including acquisition of data, interpretation and drafting/

editing of the manuscript, and have given final approval of the version to be published. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

- De U (2004) Evolution of cholecystectomy: a tribute to Carl August Langenbuch. *Indian J Surg* 66:97–100
- Mouret P (1991) From the first laparoscopic cholecystectomy to the frontiers of laparoscopic surgery: the prospective futures. *Dig Surg* 8:124
- Zhu JF, Hu H, Ma YZ, Xu MZ, Li F (2009) Transumbilical endoscopic surgery: a preliminary clinical report. *Surg Endosc* 23(4): 813–817
- Strasberg SM, Hertl M, Soper NJ (1995) An analysis of the problem of biliary injury during laparoscopic cholecystectomy. *J Am Coll Surg* 180:101
- Thurley PD, Dhingsa R (2008) Laparoscopic cholecystectomy: postoperative imaging. *AJR Am J Roentgenol* 191:794
- McGuckin M, Shea JA, Schwartz JS (1999) *Infect Control Hosp Epidemiol* 20(9):624–626
- Santos BF, Teitelbaum EN, Arafat FO, Milad MP, Soper NJ, Hungness ES (2012 Nov) Comparison of short-term outcomes between transvaginal hybrid NOTES cholecystectomy and laparoscopic cholecystectomy. *Surg Endosc* 26(11):3058–3066
- Auyang ED, Hungness ES, Vaziri K, Martin JA, Soper NJ (2009) Human NOTES cholecystectomy: transgastric hybrid technique. *J Gastrointest Surg* 13(6):1149–1150
- Oruc MT, Ugurlu MU (2013) Extraumbilical single-incision laparoscopic cholecystectomy with standard laparoscopic instruments. *Scand J Surg* 102(3):209–214
- Rao PP, Bhagwat SM, Rane A, Rao PP (2008) The feasibility of single port laparoscopic cholecystectomy: a pilot study of 20 cases. *HPB (Oxford)* 10(5):336–340
- Allemann P, Demartines N, Schäfer M (2014 Jan 21) Remains of the day: biliary complications related to single-port laparoscopic cholecystectomy. *World J Gastroenterol* 20(3):843–851
- Jørgensen LN, Rosenberg J, Al-Tayar H, Assaadzadeh S, Helgstrand F, Bisgaard T (2014 Mar) Randomized clinical trial of single- versus multi-incision laparoscopic cholecystectomy. *Br J Surg* 101(4):347–355
- Sinan H, Demirbas S, Ozer MT, Sucullu I, Akyol M (2012) Single-incision laparoscopic cholecystectomy versus laparoscopic cholecystectomy: a prospective randomized study. *Surg Laparosc Endosc Percutan Tech* 22(1):12–16
- Moher D (1998) CONSORT: an evolving tool to help improve the quality of reports of randomized controlled trials. *Consolidated Standards of Reporting Trials*. *JAMA* 279:1489–1491
- Milas M, Devedija S, Trkulja V (2014) Single incision versus standard multiport laparoscopic cholecystectomy: up-dated systematic review and meta-analysis of randomized trials. *Surgeon*
- Hernandez JM, Morton CA, Ross S, Albrink M, Rosemurgy AS (2009) Laparoendoscopic single site cholecystectomy: the first 100 patients. *Am Surg* 75(8):681–685 discussion 685–6. Erratum in: *Am Surg*. 2009;75(10):1030
- Feinberg EJ, Agaba E, Feinberg ML, Camacho D, Vemulapalli P (2012) Single-incision laparoscopic cholecystectomy learning curve experience seen in a single institution. *Surg Laparosc Endosc Percutan Tech*. 22(2):114–117

18. Geng L, Sun C, Bai J (2013 Oct 2) Single incision versus conventional laparoscopic cholecystectomy outcomes: a meta-analysis of randomized controlled trials. *PLoS One* 8(10):e76530
19. Mehmood Z, Subhan A, Ali N, Rasul S, Iqbal M, Khan R et al (2010) Four port versus single incision laparoscopic cholecystectomy. *J Surg Pakistan* 15:122–125
20. Phillips MS, Marks JM, Roberts K, Tacchino R, Onders R, DeNoto G et al (2012) Intermediate results of a prospective randomized controlled trial of traditional four-port laparoscopic cholecystectomy versus single-incision laparoscopic cholecystectomy. *Surg Endosc* 26:1296–1303

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